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# A histological survey of the brain development in the sturgeon *(Acipenser gueldenstaedtii)*

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## Abstract

Brain development of the *Acipenser gueldenstaedtii* (*A.gueldenstaedtii*) was described from days 1 to 36, post-hatch (dph) in this study. Larvae were euthanized, and serial sections of specimens were examined. Our ontogenical study was determined to concentrate on important parts of the brain containing forebrain, midbrain and hindbrain. The primary observation of the telencephalon was in 1-week-old specimens. The size of the forebrain remained relatively steady and did not demonstrate considerable changes, as compared to the other parts of brain. The diencephalon changes were noticeable in 3-week-old *A.gueldenstaedtii*. 2 major parts of mesencephalon, (tegmental portion and optic tectum) were detected during 4 weeks of development. As in other chondrosteans, the cerebellum appeared highly developed. Medulla oblongata appeared larger in 1-week-old specimens than in 4-week-old specimens.

**Keywords:** sturgeon, *Acipenser gueldenstaedtii*, development, brain, histology

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## Introduction

*Acipenser gueldenstaedtii* (*A.gueldenstaedtii*) is a valuable commercial fish species and widely distributed throughout the whole area of the Caspian Sea (Ruban and Khodorevskaya, 2011).From the appearance of sturgeon, 200 million years ago, to the present time, this group has shown both remarkable expansion and a spectacular range of evolution (Stöttinger *et al*., 2003). This evolutionary diversification is also reflected in their brain structure (Nieuwenhuys *et al*., 1998 and Va´zquez *et al*., 2002). Despite their evolutionary importance, current anatomical knowledge of the ontogenesis of the central nervous system of chondrosteans is rudimentary (Va´zquez *et al*., 2002). In chondrosteans, the main divisions of the brain - telencephalon, diencephalon, Mesencephalon and rhombencephalon can be readily distinguished (Nieuwenhuys *et al*., 1998).The telencephalon can be subdivided into three main parts: olfactory bulbs, telencephalic hemispheres, and the telencephalon. The diencephalon of sturgeon can be subdivided in epithalamus (habenula andepiphysis), thalamus, hypothalamus and pretectum. Mesencephalon is divided into a dorsal tectum and a ventral tegmentum. The cerebellum appears strongly developed and similar to other vertebrates, the cerebellar anlage evaginates dorsally during ontogenesis, leading to the formation of a cerebellar ventricular cavity (Nieuwenhuys *et al.*, 1998).Valuable previous articles include the description of the ontogenesis of the telencephalon of *Acipenser ruthenus* (Nieuwenhuys, 1964), studies on the diencephalon and pretectum of the white sturgeon (Rupp and Northcutt, 1998), investigation on development of the brain of the sturgeon *Acipense rnaccarii* (Va´zquez *et al*., 2002), and survey of brain morphology in *Acipenser stellatus* and *Acipenser persicus* (Bani *et al*., 2008). This article focuses on gross morphology and the cytoarchitecture of the sturgeon, *A.gueldenstaedtii*, brain through ontogenesis. To our knowledge, this is the first ontogenetic description of the brain of autochthonous *A.gueldenstaedtii*, from the Caspian Sea.

## Materials and methods

Samples of fish larvae (*A.gueldenstaedtii*) were obtained from Agh–Ghala Sahdi Marjani Hatchery Center, located in Gorgan, north of Iran. The acquired samples were classified in age groups of 1 to 36 days, post hatching (dph). During the following days in laboratory process, the larvae were euthanized and fixed in 10% buffered formalin, dehydrated in graded ethanol, and embedded in paraffin. Serial sections of specimens, performed with 4µm thickness, were cut sagittally or frontally from each block. Tissue sections were mounted on glass slides and stained with Hematoxylin and eosin for histological studies.

## Results

The primary observation of the telencephalon in histological specimens of *A.gueldenstaedtii* was in 1-week-old specimens (Fig. 1A). During the following weeks, the shape of the forebrain changed slowly and comparing of forebrain with other brain regions showed that considerable changes in shape of the telencephalon didn’t occur in next weeks of the development. Telencephalic ventricles were obvious and contained large areas during the four weeks of development (Figs. 1 and 2). The diencephalon was partly covered by the midbrain, and only the habenular ganglia were externally visible. Although habenular ganglia do not reveal significant changes during the first two weeks, their shapes were noticeable in 3-week-old *A.gueldenstaedtii* (Fig. 2C). Habenular ganglia were close to the telencephalic ventricles, and they contained a narrow area which is full of nuclei (Figs. 2C and 2D). The hypothalamus showed delayed development, in comparison to the other parts of the brain. The hypothalamus was considerably more apparent in specimens older than 2 weeks, and its walls were rather thin (Figs. 2C and 2D).

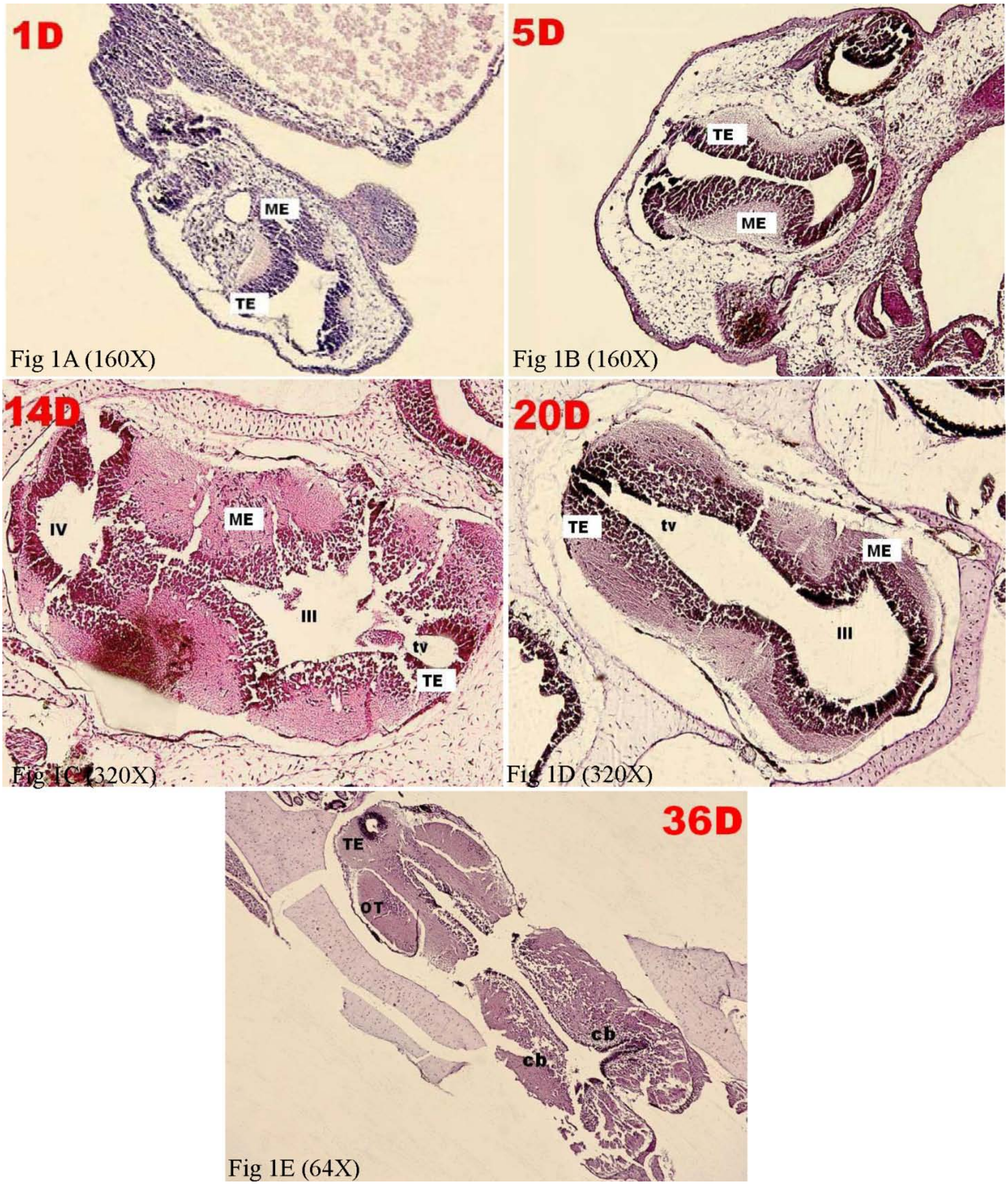



**Figure 1. Photomicrographs of Hematoxylin & eosin stained frontal sections through the brain of *Acipenser gueldenstaedtii* at different ages: 1, 5, 14, 20, and 36 days after hatching (A-E). Abbreviations: cb, cerebellum; ME, mesencephalon; OT, optic tectum; TE, telencephalon; tv, telencephalic ventricle; III, third ventricle; IV, fourth ventricle.**

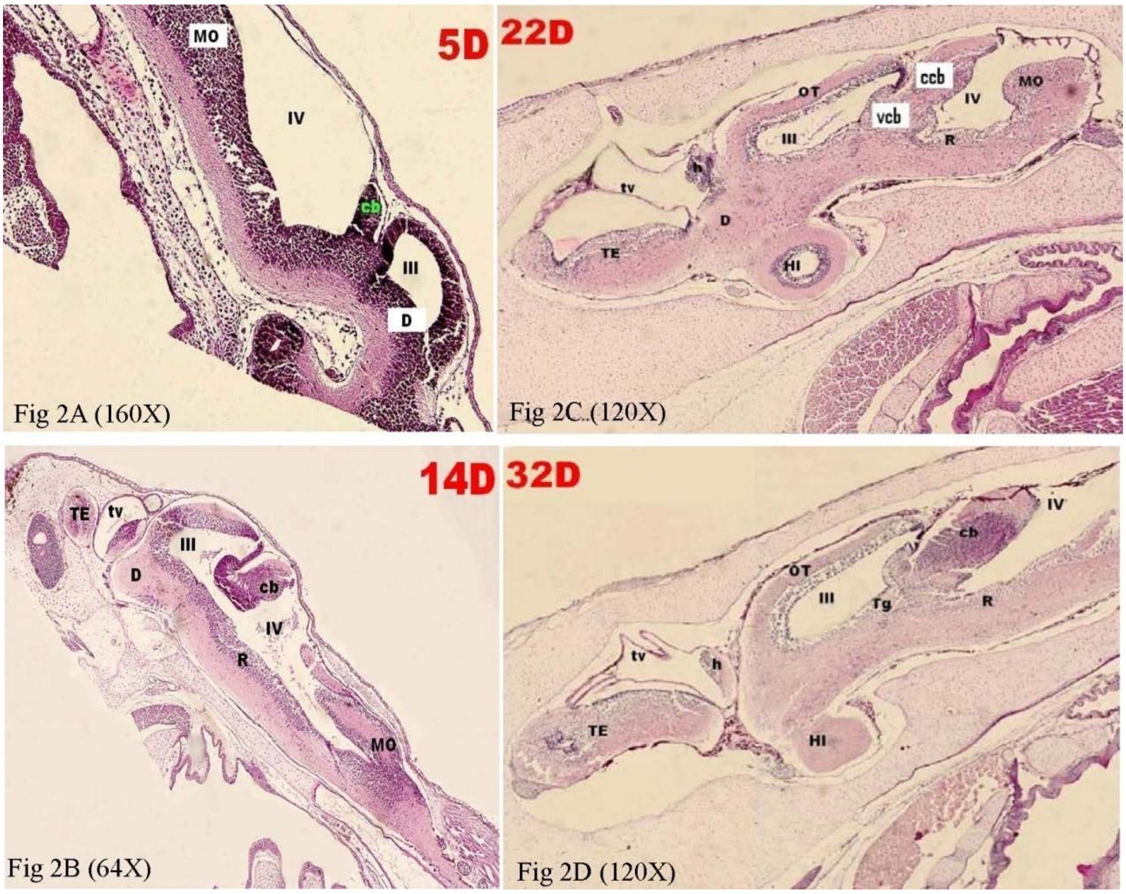



**Figure 2. Photomicrographs of Hematoxylin & eosin stained midsagittal sections of the entire brain of *Acipenser gueldenstaedtii*at 5, 14, 22 and 32 day stages (A-D). cb, cerebellum;ccb, corpus cerebelli; D, diencephalon; h, habenular ganglia; Hl, lateral hypothalamus; MO, medulla oblongata; OT, optic tectum;R, raphe nucleus; TE, telencephalon; Tg, tegmentum; tv, telencephalic ventricle;vcb, valvula cerebelli; III, Third ventricle; IV, fourth ventricle.**

The primary part of the mesencephalon, the tegmental portion, had been formed in 1-week-old specimens (Fig. 1A). The optic tectum was rather poorly developed during the early stage, and the six layers might only be differentiated in specimens that were over 40 days old. The first gross morphological appearance of the optic tectum development was seen in 2-week-old larvae (Fig. 2C). The areas containing optic tectum were above the third ventricle (Figs. 2C and 2D). The third ventricle was fairly large in young larvae, but during ontogenesis, its growth was abated (Fig. 2). Development of a duct was seen between the third and fourth ventricles, in 1-week-old specimens. As in other chondrosteans, the cerebellum appeared highly developed in *A.gueldenstaedtii* (Fig. 1D). In the juvenile cerebellum, a pair of lateral expansions and a central division had formed. The central division had a caudal part, the corpus cerebelli, and a rostral part, which expanded forward under the tectum as the valvula cerebelli. The valvula cerebelli protruded into the mesencephalic ventricular cavity (Fig. 2C). During development, changing of the cerebellum’s size was considerable, and, in 3-week-old specimens, its size was relatively large (Figs. 2C and D). Due to the presence of the purkinje cells, it was easy to distinguish the cerebellum from the other parts of the brain. Medulla oblongata which is a part of rhombencephalon was distinguished in 1-week-old larvae, and it included large areas in the caudal section of the brain (Fig. 2A). Medulla oblongata appeared larger in 1-week-

old specimens than in 4-week-old specimens (Figs. 2A and 2C). Raphe nuclei, a moderate-size cluster of nuclei under the fourth ventricle were remarkably obvious in second week after hatching (Fig. 2B). The primary development of the fourth ventricle in *A.gueldenstaedtii* was observed in 1-week-old specimens (Fig. 2A). The fourth ventricle was located between the cerebellum and medulla oblongata, and, similar to the other ventricles, its development rate diminished during ontogenesis (Figs. 1 and 2).

## Discussion

There is extensive anatomical and physiological diversity within fish species. Thus, naturally, differences will be noted in fish brains from various species. This diversity offers ample opportunity to relate ecology with brains and sensory systems. Faculties for sensory perception, central processing, and behavioral responses undoubtedly reside primarily within an organism's nervous system (Kotrschal *et al*., 1998).

As noted before in this text, the major parts of the brain studied were telencephalon, diencephalon, mesencephalon, and rhombencephalon. The anterior part of the brain is the forebrain or telencephalon, and it is responsible for learning, memory and social behaviors in fish (Broglio *et al*., 2003). In comparison to land vertebrates and other fishes, actinopterygians or ray-finned fishes have morphologically unusual forebrains (Bradford, 2009). The telencephalon can be subdivided into three main parts: olfactory bulbs, telencephalic hemispheres, and the telencephalon medium or impar (preoptic region) (Va´zquez *et al*., 2002). Throughout this study, our observations on telencephalon revealed that the primary development of the fore brain was seen in 1-week old specimens. Other studies concur, stating observation of telencephalon eversion as early as 4 days old *Acipensernaccarii* (Va´zquez *et al*., 2002). Our microscopic study demonstrated total size and shape of the forebrain during the following month remained relatively static. Other research studies, performed on different species of Acipenser, revealed considerable morphological transformation of the telencephalon occurring very late in the developmental procedure (Nieuwenhuys, 1964, Va´zquez *et al*., 2002). The differences between sturgeon fishes with other fish species may be referred to the late maturation of these valuable fishes. In other words, the physiological maturation pathways occurred over many years and, naturally, can affect brain development.

The diencephalon was the second anatomical division of our survey and, to some extent, was hidden by the midbrain. The mesencephalon and diencephalon are rostrally continuous throughout the brainstem via brain structures arising from its roof, the cerebellum, the optic tectum and the forebrain (Davis and Northcutt, 1983). Diencephalon of white sturgeon is in the intermediate level in comparison tocladistians and neoptergian fishes (Rupp and Northcutt, 1998). The diencephalon can be subdivided into the epithalamus (habenula and epiphysis), thalamus, hypothalamus, and pretectum, (Va´zquez *et al*., 2002). Results show that habenular ganglia in 3-week–old larvae have considerable shape. The nuclei of habenula are highly asymmetrical with the right side larger than left side (Rupp and Northcutt, 1998). The hypothalamus is the lower section and the largest part of the diencephalon. The inferior lobe of the hypothalamus is paired, and the ventral diencephalic hemispheres serve as multimodal integration centers (Kotrschal *et al*., 1998).

The mesencephalon or mid-brain is the center for visual sense and, in addition, is responsible for coordinating sensory signals and action responses (Kotrschal *et al*., 1998). Anatomically, the mesencephalon is rather poorly developed and maintains the embryonic tube-like shape during ontogenesis and can be divided into a dorsal tectum and a ventral tegmentum (Nieuwenhuys *et al.*, 1998 and Va´zquez *et al*., 2002). The optic tectum was

also rather poorly developed, and we were unable to distinguish the six layers. This differentiation in layers would be evident in 40-day-old larvae. Corresponding with other similar studies, the optic tectum of Acipenser species compared to polyodontidae are less highly developed (Nieuwenhuy1964, Va´zquez *et al*., 2002). According to our results, this basic development of optic tectum of mesencephalon, and thereby visual system of sturgeon species, suggests that the visual system development in these species is lower than other actinopterygian fishes (Va´zquez *et al*., 2002).Due to physiological roles of the cerebellum in the body, this part of the brain in any animal is very important, especially in fish. The cerebellum, or metencephalon, helps the fish to keep itself balanced in movements (Wagner, 2003). The size of the cerebellum varies in different fish species. In our study, a distinguishable size of the cerebellum was observed in specimens older than 3weeks. As mentioned before, a central division forms in the cerebellum, and the structure of the cerebellar central body is special only for chondrosteans (Va´zquez *et al*., 2002).The Rhombencephalon has a center for receiving different kinds of signals, including viscera sensory, somatosensory, electroreceptive, and mechanoreceptive signals, in the lateral line system (Va´zquez *et al*., 2002). The rhombencephalon of the chondrosteans is very large and can be clearly divided, bilaterally, into an alar plate and a basal plate (Nieuwenhuys *et al*., 1998).

Our survey was a preliminary study on *A.gueldenstaedtii* with histological aspects of development of the brain observed for a better understanding of sturgeon brain development. Unquestionably, more studies on these valuable and threatened species should be performed in the future.

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# بررسی بافت شناسی رشد و نمو مغز در ماهی خاویاری(تاس ماهی روسی)

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## چکیده

در این مطالعه، رشد و نمو مغز ماهی خاویاری (تاس ماهی روسی) بین سنین ۱ تا ۳۶ روزگی پس از خارج شدن از تخم، مورد بررسی بافت شناسی قرار گرفت. از لاروهای ماهی پس از مرگ با ترحم نمونه بافت شناسی تهیه شد. این مطالعه بر روی بخشهای مهم مغز متمرکز بود: مغز قدامی، مغز میانی ومغز خلفی. مشاهده اولیه تلن سفال در نمونه های بافت شناسی در سن کمتر از یک هفته بود. اندازه تلن سفال در طول رشد و نمو مغز ماهی تقریبا ثابت باقی ماند و نسبت به سایر قسمت های مغز تغییرات قابل توجه ای نشان نداد. تغییرات محسوس دراندازه دیءنسفال درسن ۳ هفتگی قابل رویت بود. ۲ قسمت اصلی ناحیه مزن سفال (قسمت تگمنتال و تکتوم بینایی) در طول ۴ هفته رشد و نمو قابل شناسایی بودند. مخچه در تاس ماهی روسی همانند سایر ماهیان استخوانی-غضروفی، کاملا رشد یافته دیده شد. ناحیه پیاز مغزی در نمونه های با سن یک هفته قسمت بیشتری از مغز را نسبت به نمونه های با سن۴ هفته اشغال کرده بود.

**واژگان کلیدی:** ماهی خاویاری، تاس ماهی روسی، رشدونمو، مغز، بافت شناسی